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TITLE: Application of FDA-Approved Memantine and Newer NitroMemantine Derivatives to Treat Neurological Manifestations in Rodent Models of Tuberous Sclerosis Complex

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14. ABSTRACT The quality of life for those afflicted by Tuberous Sclerosis Complex (TSC) is affected by intellectual and neurological disabilities mediated in part by excessive glutamatergic activity in the brain. It is important to develop rational and effective therapies for early postnatal or even embryonic treatment of these neurological manifestations. Towards this goal, we propose to investigate if administration of the FDA-approved drug, Memantine, an uncompetitive/fast off-rate antagonist of the N-methyl-D-aspartate-type glutamate receptor, and its improved derivative, NitroMemantine, ameliorate neurological complications in mouse models of TSC. During Year 01 of the grant, we obtained favorable results for Memantine on electrophysiological and neurobehavioral tests in <i>Tsc2</i> <sup>+/-</sup> mice. Treatment of <i>Tsc2</i> <sup>+/-</sup> mice with Memantine improved long-term potentiation (LTP), an electrical correlate of learning and memory, in the CA1 region of the hippocampus. Additionally, treatment of <i>Tsc2</i> <sup>+/-</sup> mice with Memantine showed a trend towards restoration of the ability to locate the platform in the Morris water maze, a neurobehavioral test of hippocampal memory. In Year 02, we plan to further validate and extend our findings with Memantine and critically test the efficacy of NitroMemantine. We expect that NitroMemantine will offer an advantage over Memantine in TSC model mice, as we have previously demonstrated in other neurological disorders.					
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## 1. INTRODUCTION:

Tuberous sclerosis complex (TSC) is caused by heterozygous mutations that inactivate one of two genes, *TSC1* or *TSC2*. The central nervous system is affected in TSC, and neurological manifestations include intellectual disabilities, neurobehavioral abnormalities such as autism, and epilepsy. Currently, there is no cure for TSC. The mechanism of pathogenesis is not entirely clear, but TSC-related neurological symptoms are accompanied by excessive glutamatergic activity and altered synaptic spine structures. Our group previously developed, characterized, and licensed patents for the drug Memantine (Namenda®), which is now FDA approved for treatment of moderate-to-severe Alzheimer's disease, showing that it is an uncompetitive/fast off-rate antagonist of the *N*-methyl-D-aspartate-type glutamate receptor (NMDAR). Interestingly, Memantine is also in clinical trials for children with epilepsy, intellectual disabilities, and autism. Additionally, we have recently developed a new series of derivatives, termed NitroMemantines, with superior efficacy to Memantine both *in vitro* and *in vivo*. Our recent work has demonstrated that Memantine and NitroMemantine selectively block excessive activity of extrasynaptic NMDARs (eNMDARs) and protect synaptic integrity. Furthermore, our phosphoproteomic analysis of eNMDAR-mediated signaling has identified upregulation of the p38 MAPK (mitogen-activated protein kinase)-MAPKAPK2 (mitogen-activated protein kinase-activated protein kinase 2) cascade. Since this pathway is known to inactivate TSC2, inhibition of eNMDARs with Memantine or NitroMemantine should increase TSC2 activity. Therefore, we hypothesized that this action of Memantine and NitroMemantine may offer a rational treatment for individuals with TSC. To begin to test this hypothesis, we thus proposed the following specific aim:

1. To investigate if Memantine or NitroMemantine administration improves synaptic spine structure, electrophysiological properties, and behavioral abnormalities in TSC murine models by affecting the eNMDAR/p38 MAPK/ MAPKAPK2/TSC2 cascade.

We proposed to examine if excessive eNMDAR activity and its downstream signaling cascade, which inhibits TSC2 activity, is a pathogenic mechanism in the development of synaptic spine abnormalities, electrophysiological abnormalities, and neurobehavioral manifestations in TSC. Also, we are investigating if the FDA-approved drug Memantine or the new improved derivative that we have synthesized, NitroMemantine, can alleviate synaptic spine and behavioral abnormalities in TSC murine models. Thus, this proposal will yield further mechanistic insight into the disease and evaluate new potential pharmaceutical approaches to treatment.

## 2. KEYWORDS:

Epilepsy, Intellectual disability, Autism, Excessive glutamatergic activity, Extrasynaptic NMDA receptors, Memantine, Uncompetitive antagonist of NMDA receptors, p38MAPK, MAPKAPK2, mTOR, p70 S6 kinase

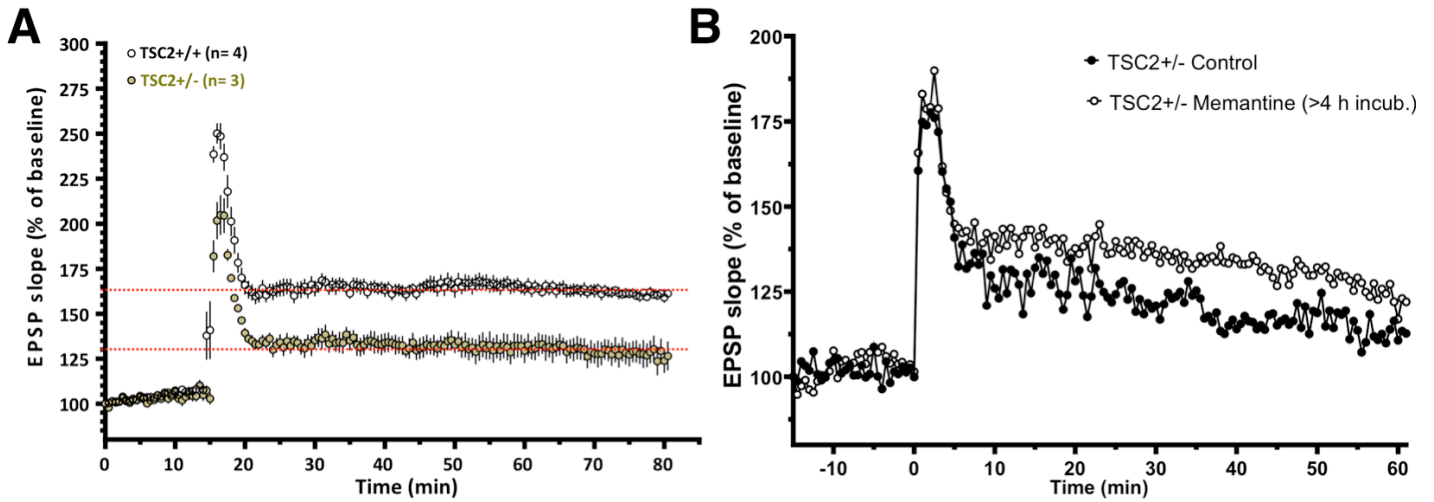
## 3. OVERALL PROJECT SUMMARY:

Task 1. We will test the effect of Memantine vs. NitroMemantine in the *Tsc1*<sup>+/-</sup> and *Tsc2*<sup>+/-</sup> mouse models of TSC, including electrophysiological, histological, biochemical, and behavioral readouts (months 1-24)

## Results

To fulfill the aim of this grant, we had proposed to obtain *Tsc2* knockout mice from Jackson Laboratory. However, since the Jackson Laboratory possessed only frozen embryos, it was expected to take several months to have *Tsc2* knockout mice from that source. Thus, we instead obtained the *Tsc2*<sup>+/+</sup> and *Tsc2*<sup>+/-</sup> mice from Dr. Mark F. Bear (MIT) (1). We initially had technical difficulties in genotyping following Dr. Bear's protocol that caused a delay of a couple of months before being able to begin breeding for the proposed experiments. As soon as we obtained *Tsc2*<sup>+/+</sup> and *Tsc2*<sup>+/-</sup> mice from our breeding colony, we first examined LTP (long-term potentiation) using field recordings in the CA1 region of hippocampal slices to investigate the effects of these mutations on synaptic plasticity. We prepared acute hippocampal slices from one-month old mice. We performed field recording in an MEA (microelectrode array) chamber perfused with ACSF (artificial cerebrospinal fluid). We recorded fEPSPs (field excitatory postsynaptic potentials) in the CA1 region with the MEA. We evoked LTP by stimulation of the Schaffer collaterals (four repetitions of 100 Hz pulses for one sec each). The initial slope of the fEPSP was analyzed to assess synaptic plasticity. As shown in **Fig. 1A** (see next page), we confirmed abnormality of LTP in *Tsc2*<sup>+/-</sup> mice (2). Next, we found that treatment with 10  $\mu$ M

Memantine over 4 hours produced a beneficial effect (**Fig. 1B**). Based on this result, we are now optimizing the dose and timing of Memantine treatment, and will then utilize these optimized conditions to compare statistically the effects of Memantine versus NitroMemantine on improving LTP.

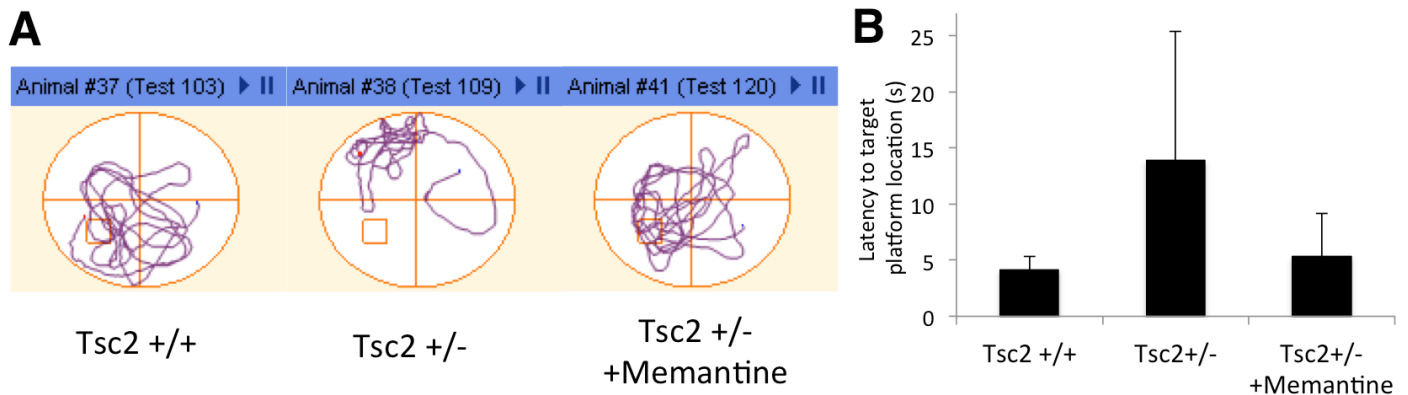


**Fig. 1. Abnormal CA1-LTP in  $Tsc2^{+/-}$  mice**

(A) LTP recorded from hippocampal slices by MEA. Data are plotted every 30 sec and represent means  $\pm$  s.e.m.  $Tsc2^{+/+}$ ,  $n = 4$  slices total from 4 mice;  $Tsc2^{+/-}$ ,  $n = 3$  slices total from 3 mice.

(B) Effect of Memantine treatment (10  $\mu$ M incubation over 4 hours) on LTP in  $Tsc2^{+/-}$  mice. ‘Control’ refers to treatment with vehicle rather than Memantine.

Next, to explore the effects of Memantine on the learning and memory deficits in  $Tsc2^{+/-}$  mice (2), we performed a behavioral readout, the Morris water maze test on three month-old  $Tsc2^{+/+}$  and  $Tsc2^{+/-}$  mice, as we had proposed. We administered Memantine intraperitoneally each day, 2 hours before the training sessions for that day. Mice received four training sessions per day for 5 consecutive days. We assessed spatial learning with a probe trial, during which mice swam for 60 sec. **Fig. 2A** shows representative tracking plots during the probe test. This initial set of tests demonstrated a strong trend that Memantine-treated  $Tsc2^{+/-}$  mice take a shorter time to reach the original platform location than do vehicle-treated  $Tsc2^{+/-}$  mice (**Fig. 2B**). In the coming grant year, we plan to increase the number of animals tested to see if this finding reaches statistical significance, as we had hypothesized. We will also determine the effects of the improved derivative,



**Fig. 2. Effect of Memantine treatment on the Morris water maze in  $Tsc2^{+/-}$  mice**

(A) Representative paths during a 60 sec probe test

(B) Time to reach the target platform location. Values are means  $\pm$  s.e.m.;  $n = 8$  mice for  $Tsc2^{+/+}$ ,  $n = 3$  mice for  $Tsc2^{+/-}$ ,  $n = 3$  mice for  $Tsc2^{+/-}$  + Memantine.

NitroMemantine, on the Morris water maze test in *Tsc2*<sup>+/-</sup> mice. For histological and molecular evaluation, we are collecting brain samples after the Morris water maze test to examine synaptic density and biochemical activity of the eNMDAR/p38 MAPK/MAPKAPK2/TSC2/mTORC1 cascade, as we had proposed in the original application.

### **Progress and Accomplishments with Discussion**

The goal of this project is to determine if administration of the FDA-approved drug Memantine and its improved derivative, NitroMemantine, mitigates neurological manifestations in mouse models of TSC. A technical issue in genotyping of TSC mice caused a delay in the initiation of the experiments. However, once up and running, during Year 01 of the grant, we obtained favorable effects of Memantine on electrophysiology and behavior in *Tsc2*<sup>+/-</sup> mice, as described above in the Results section. In Year 02, we plan to further validate and extend our findings on Memantine by utilizing electrophysiological, histological, biochemical, and behavioral approaches as proposed in the original grant application. Additionally, we will test the efficacy of NitroMemantine, as we had proposed in the original application. We expect that NitroMemantine will offer additional benefit over Memantine in TSC model mice because we recently found that NitroMemantine provided synaptic protection and rescued behavioral abnormalities to a greater extent than Memantine in Alzheimer's mouse models (3).

### **4. KEY RESEARCH ACCOMPLISHMENTS**

- Memantine treatment can restore synaptic plasticity in *Tsc2*<sup>+/-</sup> mice, as monitored by recording LTP.
- Memantine treatment shows promise in ameliorating deficits in learning and memory in *Tsc2*<sup>+/-</sup> mice, as monitored in the Morris water maze neurobehavioral test.

### **5. CONCLUSION**

The results from this proposal will have a major impact on medical treatment because they have the potential to initiate new treatments for the neurological manifestations of TSC, which are thought to be mediated in part by excessive glutamatergic activity in the brain. To this end, we are testing the FDA-approved drug Memantine, which our group played a major role in developing for other neurological indications, as well as our improved derivatives, termed NitroMemantines. Since the quality of life for those with TSC is most affected by intellectual and neurological disabilities, it is important that we understand the pathogenesis of early cognitive deficits in order to develop rational and effective therapies for early postnatal or even embryonic treatment of TSC. Hence, we are hopeful that children with TSC will benefit from the research proposed here via development of new drug therapies to increase their cognitive capacity and mitigate neurobehavioral abnormalities. Our initial results from treating *Tsc2*<sup>+/-</sup> mice demonstrate promising beneficial effects. During Year 02, we plan to confirm and extend our findings with Memantine and also test the efficacy of the improved analog, NitroMemantine.

### **6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:**

Nothing to report.

### **7. INVENTIONS, PATENTS, AND LICENSES:**

Nothing to report.

### **8. REPORTABLE OUTCOMES;**

Treatment of TSC2 heterozygote mice with 10  $\mu$ M Memantine for 4 hours improved long-term potentiation (LTP) in the CA1 region of the hippocampus. Previously, it had been shown that LTP, an electrical correlate of learning and memory, was decreased in TSC2 heterozygote mice. We now report that treatment with Memantine (Namenda®), an FDA—approved drug that had been developed in our laboratory and licensed to Forest Laboratories in NYC, resulted in an increase in LTP in these mice. Additionally, TSC2 heterozygous mice show a decrement in Morris water maze activity, a neurobehavioral test of hippocampal memory. Treatment with Memantine showed a trend towards restoration of normal memory in these mice. In Year 02,

larger cohorts of mice and optimization of the treatment paradigm are planned in order to perform a rigorous statistical analysis of the results of these memory tests.

## **9. OTHER ACHIEVEMENTS:**

Nothing to report.

## **10. REFERENCES:**

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## **11. APPENDICES:**

N/A